

# Free Energies of Hydration Using Restrained Electrostatic Potential Derived Charges via Free Energy Perturbations and Linear Response

RICHARD H. HENCHMAN, JONATHAN W. ESSEX

*Department of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, United Kingdom*

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**ABSTRACT:** To determine the reliability of the restrained electrostatic potential derived (REPD) charge method described in a companion article, the free energies of hydration of 22 simple organic molecules were calculated using free energy perturbations and linear response theory. Of the four charge sets examined using the free energy perturbation method, REPD/6–31<sup>+</sup>G\* charges gave the closest agreement with the experiment, followed by conventional EPD charges derived at the 6–31G\* level. The average absolute error for the former was 2.9 kJ mol<sup>−1</sup> and for the latter was 3.5 kJ mol<sup>−1</sup>. The linear response method was then used to predict free energies of hydration based on the average van der Waals and electrostatic solute-solvent energies obtained from Monte Carlo computer simulations and solvent accessible surface areas. Use of the three parameter relationship gave a good agreement with the experiment. However, a number of simpler fitting functions were also examined and found to be only marginally less reliable. A careful statistical analysis of the linear response method is therefore needed. © 1999 John Wiley & Sons, Inc. *J Comput Chem* 20: 499–510, 1999

**Keywords:** free energy; free energy perturbation; linear response; OPLS; electrostatic potential

## Introduction

In the companion article, pages 483–498 of this issue, we proposed a method for generating atomic charges that are optimized to their equivalent OPLS values.<sup>1</sup> This procedure involves the fitting of charges to the molecular electrostatic potential (MEP) calculated from the *ab initio* wave function together with the application of a restraint to reduce the magnitude of the charges based on the method of Bayly et al.<sup>2</sup> These restrained EP derived (REPD) charges calculated at either the HF/6–31G\* or HF/6–31<sup>+</sup>G\* level are able to reproduce OPLS charges with identical correlation coefficients of 0.97, which is an improvement on unrestrained EPD charges (correlation coefficient 0.93). Because REPD charges are intended for use in condensed phase computer simulations, it is imperative that the charges are able to reproduce experimental condensed phase data. Therefore, the commonly used technique of calculating a molecule's free energy of hydration and comparing it with an experiment is employed here to examine their performance. It is already known that the free energies of hydration of molecules using EPD/6–31G\* charges compare well with experimental values with an average error of around 4 kJ mol<sup>–1</sup>.<sup>3–5</sup> The effect of the restraint and basis set is studied in this work by calculating the free energies of hydration for EPD and REPD charges using 6–31G\* and 6–31<sup>+</sup>G\* basis sets.

Free energies of hydration are calculated using two different methods. The free energy perturbation (FEP) method works best for small transformations and consequently requires a large number of simulations between the initial and final states of the mutation for a precise free energy to be determined. Owing to the computationally intensive nature of this method, an alternative procedure was also tested: linear response (LR) theory.<sup>6–8</sup> This method has the advantage of only requiring simulations at the end points of a mutation but has the disadvantage of being an approximate, empirical method requiring parameterization to known free energy data obtained from either an experiment or simulation.

## Method

The free energies of hydration are calculated for the 22 molecules listed in Table I using both FEP

and LR in Monte Carlo computer simulations. The suitability of the rigid molecule approximation was the main criterion for choosing these molecules from the original 29 used in the REPD parameterization discussed in our previous article.<sup>1</sup> Thus, most molecules contain no significant conformational degrees of freedom, apart from those associated with hydrogens on methyl groups. Of those molecules with conformational degrees of freedom, methyl acetate, acetic acid, phenol, and aniline were included to ensure that all chemical functionalities were represented. In addition, acetamide and *trans*-N-methyl acetamide were included because the barrier to rotation about the amide C–N bond is large enough to justify rigidity. Benzonitrile was excluded because it lacked an experimental free energy of hydration, while formamide and formaldehyde were retained due to their usefulness as intermediates in the free energy calculations. The 6–31G\* and 6–31<sup>+</sup>G\* optimized geometries used in the REPD parameterization were adopted for the simulations. Standard OPLS Lennard–Jones parameters were used.<sup>9</sup> The atomic charges used were those developed in our previous article<sup>1</sup>: EPD/6–31G\*, REPD/6–31G\*, EPD/6–31<sup>+</sup>G\*, and REPD/6–31<sup>+</sup>G\*. The simulations were performed in TIP4P water.<sup>10</sup>

To calculate the free energy of hydration for a rigid molecule, the molecule is mutated from itself to nothing in aqueous solution. Using FEP, the free energy difference between two states *i* and *j* is given by

$$\Delta G_{ij} = -RT \ln \langle \exp(-\Delta \mathcal{H}_{ij}/RT) \rangle_i. \quad (1)$$

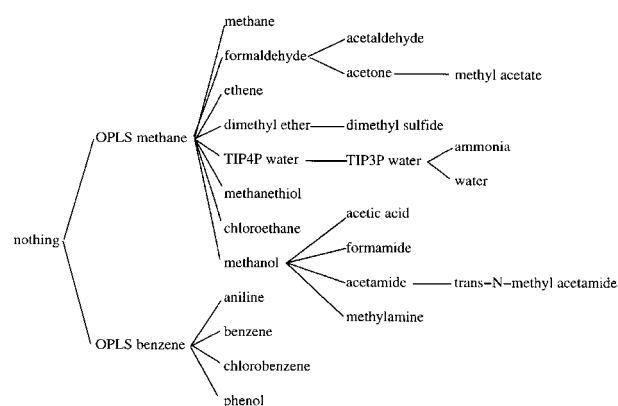
This equation is evaluated by calculating the change in the Hamiltonian,  $\Delta \mathcal{H}_{ij}$ , for perturbing from state *i* to state *j* and performing the average over the equilibrium configurations for state *i*.<sup>11</sup> The reverse free energy may be evaluated by calculating the change in the  $\Delta \mathcal{H}_{ji}$  and averaging over equilibrium configurations for state *j*. That one should be the negative of the other provides a consistency check that the average in eq. (1) has converged. However, in practice, this average converges satisfactorily only if the two states are very similar. Therefore, the mutation to nothing is divided into a number of smaller stages. First the molecules are mutated to their next simplest most similar molecule as shown in the mutation “tree” in Figure 1. By calculating the free energy changes for all the stages in the tree, the absolute free energy for any molecule is then calculated by

**TABLE I.**  
**Calculated Free Energies of Hydration ( $\text{kJ mol}^{-1}$ ) Using FEP versus Experiment<sup>19–22</sup> for All Four Charge Sets.**

Molecule	Experiment	$\Delta G(\text{EPD} / 6-31\text{G}^*)$	$\Delta G(\text{REPD} / 6-31\text{G}^*)$	$\Delta G(\text{EPD} / 6-31^+\text{G}^*)$	$\Delta G(\text{REPD} / 6-31^+\text{G}^*)$
Methane	8.4	$9.0 \pm 0.8$	$9.1 \pm 0.8$	$9.0 \pm 0.8$	$9.1 \pm 0.8$
Ethene	5.3	$6.0 \pm 1.0$	$6.4 \pm 1.0$	$4.9 \pm 1.0$	$5.6 \pm 1.0$
Water	–26.4	$-22.0 \pm 1.3$	$-20.7 \pm 1.3$	$-26.4 \pm 1.4$	$-24.7 \pm 1.3$
Methanol	–21.4	$-18.3 \pm 1.2$	$-16.5 \pm 1.2$	$-22.8 \pm 1.4$	$-19.8 \pm 1.2$
Methanethiol	–5.2	$-1.9 \pm 1.2$	$-1.7 \pm 1.2$	$-2.5 \pm 1.2$	$-2.2 \pm 1.2$
Acetaldehyde	–14.7	$-14.2 \pm 1.7$	$-12.9 \pm 1.7$	$-18.6 \pm 1.7$	$-16.6 \pm 1.7$
Acetone	–16.1	$-14.5 \pm 1.8$	$-12.8 \pm 1.8$	$-19.2 \pm 1.9$	$-17.0 \pm 1.8$
Acetic acid	–28.1	$-31.8 \pm 2.7$	$-27.8 \pm 2.7$	$-36.0 \pm 2.8$	$-30.0 \pm 2.7$
Methyl acetate	–13.9	$-18.9 \pm 2.0$	$-15.9 \pm 1.9$	$-22.4 \pm 2.1$	$-18.0 \pm 1.9$
Ammonia	–18.0	$-10.7 \pm 1.6$	$-12.7 \pm 1.6$	$-15.3 \pm 1.6$	$-12.3 \pm 1.6$
Methylamine	–19.1	$-19.7 \pm 1.4$	$-16.2 \pm 1.3$	$-22.2 \pm 1.3$	$-17.2 \pm 1.3$
Acetamide	–40.6	$-43.1 \pm 3.6$	$-36.6 \pm 3.5$	$-49.8 \pm 3.6$	$-40.9 \pm 3.5$
<i>trans</i> -N-Methyl acetamide	–42.2	$-38.8 \pm 3.8$	$-35.4 \pm 3.8$	$-43.8 \pm 3.9$	$-39.5 \pm 3.8$
Dimethyl ether	–7.9	$-3.0 \pm 1.4$	$-2.9 \pm 1.4$	$-4.5 \pm 1.4$	$-4.7 \pm 1.4$
Dimethyl sulfide	–6.4	$1.6 \pm 1.7$	$1.6 \pm 1.7$	$1.3 \pm 1.7$	$1.4 \pm 1.7$
Chloroethane	–2.6	$1.7 \pm 2.2$	$1.7 \pm 2.2$	$1.7 \pm 2.2$	$1.7 \pm 2.2$
Benzene	–3.6	$-7.8 \pm 1.7$	$-7.6 \pm 1.7$	$-11.5 \pm 1.7$	$-10.8 \pm 1.7$
Phenol	–27.7	$-27.0 \pm 2.0$	$-23.8 \pm 2.0$	$-30.1 \pm 2.0$	$-25.2 \pm 2.0$
Aniline	–20.5	$-26.3 \pm 1.8$	$-19.5 \pm 1.7$	$-35.9 \pm 1.8$	$-19.4 \pm 1.7$
Chlorobenzene	–4.7	$-9.7 \pm 1.9$	$-9.5 \pm 1.9$	$-9.7 \pm 1.9$	$-9.4 \pm 1.9$
Formaldehyde	—	$-11.3 \pm 1.3$	$-10.6 \pm 1.3$	$-14.3 \pm 1.3$	$-13.5 \pm 1.3$
Formamide	—	$-40.4 \pm 2.0$	$-34.6 \pm 1.9$	$-46.6 \pm 2.0$	$-38.2 \pm 2.2$
Average unsigned error <sup>a</sup>		3.5	3.7	4.6	2.9

<sup>a</sup> With respect to the experiment.

summing the components. In this work all molecules were eventually mutated to OPLS methane, OPLS benzene, or TIP4P water, for which the free energies of mutating to nothing were already determined.<sup>12–14</sup> To further ensure reliable convergence, each mutation between molecules was subdivided into a number of windows de-



**FIGURE 1.** Free energy tree showing the mutations performed to calculate the free energies of hydration.

fined by the coupling parameter  $\lambda$ , which varies from 0 to 1. Geometries and nonbonded parameters scale linearly with  $\lambda$  between the initial and final molecules. At each value of  $\lambda$ , a simulation was performed and the free energy calculated for mutating to the next and previous window using eq. (1). The free energy change was taken as the average of the forward and reverse free energies. For mutations in which no atoms were destroyed, six  $\lambda$  windows were used that were spaced at 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0. However, additional windows were used in other cases in which significant hysteresis of  $1 \text{ kJ mol}^{-1}$  was evident for a given window, particularly where methyl groups were mutated to hydrogens and where polar groups were adjusted. The mutations for *trans*-N-methyl acetamide and methanol were broken up into two completely separate simulations for which the geometry and then the nonbonded parameters were perturbed. Statistical errors for each  $\lambda$  window were calculated as half the difference between the forward and reverse free energy changes; the total error for the mutation was then the square root of

the sum of squares of the individual errors for each window. The mutations between molecules as given in Figure 1 were carried out using the REPD/6-31G\* charge set. The free energies for the remaining charge sets were calculated by mutating to their respective REPD/6-31G\* molecules in three  $\lambda$  windows of 0.0, 0.5, and 1.0.

Free energy changes were calculated using BOSS 3.6.<sup>15</sup> Each solute molecule was placed in a cubic cell with sides of 26.6 Å containing 648 water molecules. To ensure faster equilibration, 7–10 water molecules with the highest interaction energy with the solute were discarded, depending on the solute size. Equilibrium configurations were generated in the isothermal-isobaric ensemble at 25°C and 1 atm using the Metropolis algorithm and preferential sampling.<sup>16</sup> Periodic boundary conditions and a nonbonded cutoff of 10 Å based on the distance between the water oxygen atom and any solute atom were used. The potentials were quadratically feathered to zero over the last 0.5 Å. At each  $\lambda$  window the system was equilibrated for 3 million (M) configurations followed by 5 M configurations of data collection. Solute moves were attempted every 100 configurations and volume changes every 2500 configurations. The ranges for solute translation and rotation moves were selected to be between 0.1 and 0.3 Å and 10 and 30°, respectively, to give an acceptance probability of approximately 40%. As a test for the convergence of the free energies, some of the mutations involving the more polar molecules were extended to 10 M configurations of data collection.

The LR free energies of hydration may be calculated using the equation<sup>7</sup>

$$\Delta G = \alpha \langle U_{\text{vdW}} \rangle + \beta \langle U_{\text{elec}} \rangle + \gamma (\text{SASA}), \quad (2)$$

where  $\langle U_{\text{elec}} \rangle$  and  $\langle U_{\text{vdW}} \rangle$  are the solute–solvent electrostatic and van der Waals energies, respectively, averaged over equilibrium configurations of the molecule in water and the SASA is the solvent accessible surface area calculated using a solvent radius of 1.4 Å. Because the solutes were held rigid in these calculations, the SASA was constant for each solute over the simulation. The parameters  $\alpha$ ,  $\beta$ , and  $\gamma$  were optimized using a subplex algorithm<sup>17</sup> to minimize the absolute difference between eq. (2) and either FEP or experimental free energies summed over all molecules in the fitting set. The fitting sets used were the molecules for each of the four charge sets individually and then all four charge sets combined. The simulation protocol was identical to that applied

in the FEP calculations.  $\langle U_{\text{elec}} \rangle$  and  $\langle U_{\text{vdW}} \rangle$  were calculated from the same 5 M configurations used for the first window of that molecule's FEP simulation. SASAs were calculated using Macromodel.<sup>18</sup> Formaldehyde and formamide were excluded from the fits to the experiment because the experimental data were not available but were included in the fit to FEP. Two studies were carried out to determine the sensitivity of the LR parameters to the molecular quantities and molecules used in the parameterizations: a cross-validation analysis and a randomization analysis. The results of these studies raised the possibility that the LR equation used [eq. (2)] was overfitting, and so a range of simpler LR functions were also examined.

## Results

### FEP

The free energies of hydration calculated by FEP are presented with simulation errors in Table I for all 22 molecules for each of the four charge sets, together with the experimental values.<sup>19–22</sup> The average unsigned error for 20 molecules with respect to the experiment given at the bottom of the table indicates the overall performance for that charge set. To further clarify the comparison with the experiment, Table II contains the errors with respect to the experiment for each molecule and charge set. The average signed error is given at the bottom of this table and indicates whether the free energies are on average too positive or too negative compared with the experiment. The longer simulations consisting of 10 M configurations of data collection gave smaller errors as expected. Also, the calculated free energies were identical to those obtained over 5 M configurations of data collection to within error. For example, the acetamide to methanol mutation changed from  $18.3 \pm 3.3$  to  $20.1 \pm 2.4$  kJ mol<sup>−1</sup>; the methanol to methane mutation changed from  $24.8 \pm 0.8$  to  $25.6 \pm 0.8$  kJ mol<sup>−1</sup>; and the acetic acid to methanol mutation was unchanged at 11.3 kJ mol<sup>−1</sup>, the error decreasing from  $\pm 2.4$  to  $\pm 1.5$  kJ mol<sup>−1</sup>. Thus, 5 M configurations of data collection is sufficient for the performance of charge sets to be determined. From Tables I and II it can be seen that the REPD/6-31<sup>+</sup>G\* charges perform the best with an average absolute error of only 2.9 kJ mol<sup>−1</sup>. The commonly used EPD/6-31G\* results are the next most reliable with an average absolute error of 3.5 kJ mol<sup>−1</sup>. The REPD/6-31G\* and

**TABLE II.** Errors in  $\Delta G_{\text{hyd}}$  (kJ mol<sup>-1</sup>) with Respect to Experiment for All 20 Molecules Using All Four Charge Sets.

Molecule	$q(\text{EPD} / 6-31\text{G}^*)$	$q(\text{REPD} / 6-31\text{G}^*)$	$q(\text{EPD} / 6-31^+\text{G}^*)$	$q(\text{REPD} / 6-31^+\text{G}^*)$
Methane	0.6	0.7	0.6	0.7
Ethene	0.7	1.1	-0.4	0.3
Water	4.4	5.7	0.0	1.7
Methanol	3.1	4.9	-1.4	1.6
Methanethiol	3.3	3.5	2.7	3.0
Acetaldehyde	0.5	1.8	-3.9	-1.9
Acetone	1.6	3.3	-3.1	-0.9
Acetic acid	-3.7	0.3	-7.9	-2.1
Methyl acetate	-5.0	-2.0	-8.5	-4.1
Ammonia	7.3	5.3	2.7	5.7
Methylamine	-0.6	2.9	-3.1	1.9
Acetamide	-2.5	4.0	-9.2	-0.3
<i>trans</i> -N-Methyl acetamide	3.4	6.8	1.6	2.7
Dimethyl ether	4.9	5.0	3.4	3.2
Dimethyl sulfide	8.0	8.0	7.7	7.8
Chloroethane	4.3	4.3	4.3	4.3
Benzene	-4.2	-4.0	-7.9	-7.2
Phenol	0.7	3.9	-2.4	2.5
Aniline	-5.8	1.0	-15.4	1.1
Chlorobenzene	-5.0	-4.8	-5.0	-4.7
Average error	0.8	2.6	-2.4	0.8

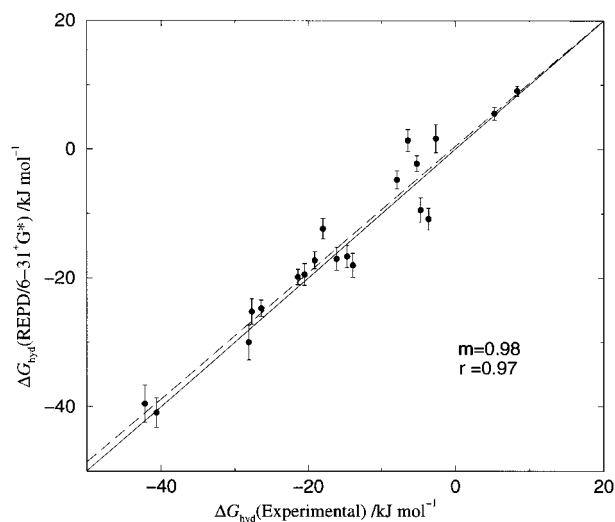
EPD/6-31<sup>+</sup>G\* charge sets performed less satisfactorily, the former being too hydrophobic and the latter too hydrophilic. Evidently, application of the restraint makes the free energies of hydration *less* negative while changing from the 6-31G\* to the 6-31<sup>+</sup>G\* basis set makes them *more* negative. For the REPD/6-31<sup>+</sup>G\* charge set these effects largely cancel, producing free energies with a similar average signed error to EPD/6-31G\* but with an improved average absolute error. Figure 2 gives a clear indication of the good correlation between REPD/6-31<sup>+</sup>G\* and experimental free energies of hydration. The slope for the line of best fit is 0.98 and the correlation coefficient is 0.97. The slope

and correlation data for the other three charge sets may be found in Table III.

The results presented are an improvement on those of Carlson et al.<sup>3</sup> who calculated an average error of 4.4 kJ mol<sup>-1</sup> for the 13 molecules used in

**TABLE III.** Slopes (*m*) and Correlation Coefficients (*r*) for  $\Delta G_{\text{hyd}}$  of All Four Charges Sets Versus Experiment.

Charge Set	<i>m</i>	<i>r</i>
EPD / 6-31G*	1.02	0.96
REPD / 6-31G*	0.91	0.97
EPD / 6-31 <sup>+</sup> G*	1.15	0.95
REPD / 6-31 <sup>+</sup> G*	0.98	0.97

**FIGURE 2.** FEP REPD / 6-31<sup>+</sup>G\* free energies of hydration versus the experiment for the molecules listed in Table I. (---) The line of best fit and the (—) unit slope.

their calculations. In that study the EPD/6-31G\* charges were used with OPLS geometries. To allow a more valid comparison, the nine molecules used in this study and the work of Carlson et al.<sup>3</sup> were examined. In this work the average error using the nine common molecules was 3.2 kJ mol<sup>-1</sup> for EPD/6-31G\* and 2.7 kJ mol<sup>-1</sup> for REPD/6-31<sup>+</sup>G\*, an improvement on the Carlson et al.<sup>3</sup> result of 4.9 kJ mol<sup>-1</sup>. There are a number of possible reasons for this difference. These include the use of different geometries, point selection schemes, and FEP simulation protocols. As an example of the importance of geometry, 6-31G\* optimized acetamide with EPD/6-31G\* charges was mutated to the OPLS geometry with EPD/6-31G\* charges rederived for that geometry. The free energy for this mutation was calculated to be 6 kJ mol<sup>-1</sup>. Part of this may be attributed to the increase in dipole moment from 4.04 to 4.38 D for the OPLS geometry. In general, however, given the small number of molecules considered and the detrimental effect a few poorly reproduced molecules can have on the overall average, more molecules would need to be considered and a more systematic investigation undertaken to ascertain whether the difference between these studies is significant.

For a number of molecules studied here, the calculated free energies of hydration are rather inaccurate, irrespective of charge set. REPD/6-31<sup>+</sup>G\* molecules with an error greater than 4 kJ mol<sup>-1</sup> are methyl acetate, benzene, and chlorobenzene, which are too hydrophilic, and ammonia, dimethyl sulfide, and chloroethane, which are too hydrophobic. The charge parameterization seems to be wanting for third row atoms such as sulfur and chlorine. All of the charges listed<sup>1</sup> for sulfur and chlorine deviate significantly from OPLS values, but there is no consistent trend between  $\Delta G_{\text{hyd}}$  and charge. The rigid molecule approximation may be responsible for the discrepancy for a number of larger molecules such as methyl acetate. Benzene is an especially interesting case. Because of symmetry, only one parameter is necessary to describe its electrostatic properties within the point charge approximation, making this number particularly critical. A trend is apparent by comparing the benzene free energies with charges. The EPD/6-31G\* value in Table I, the OPLS value,<sup>13</sup> and the value of Carlson et al.<sup>3</sup> are -7.8, -3.8, and -1.7 kJ mol<sup>-1</sup>, respectively, while the corresponding charges are -0.133, -0.115, and -0.103; it is clear that benzene's free energy of hydration scales rather sensitively with its carbon charge. A

difference of 0.016 in charge between EPD/6-31G\* and EPD/6-31<sup>+</sup>G\* benzene results in a large change in free energy of 3.7 kJ mol<sup>-1</sup>. This sensitivity is especially important given the strong dependence of benzene's EPD charges on point selection as discussed in our accompanying article.<sup>1</sup> Fortunately, when the symmetry of benzene is lowered as in aniline and phenol, the agreement with the experiment improves. The disagreement for ammonia is perplexing given the success for water: it may conceivably be related to geometry, the accuracy of which is especially important for small, polar molecules. The above-mentioned discrepancies may also be due to the Lennard-Jones parameters and even the form of the force field itself. If these problem molecules are removed, the average error for REPD/6-31<sup>+</sup>G\* molecules reduces to only 1.7 kJ mol<sup>-1</sup>, which is significantly better than the corresponding error of 2.6 kJ mol<sup>-1</sup> for EPD/6-31G\*. A number of workers have noted that free energy calculations fail to reproduce the increase in hydrophilicity observed experimentally for acetamide and ammonia when a hydrogen on the nitrogen is replaced by a methyl group.<sup>4,23</sup> The results of this work are no different for acetamide and *trans*-N-methyl acetamide. However, for the REPD/6-31<sup>+</sup>G\* charge set, the relative free energy difference was calculated to be incorrect by only  $3.0 \pm 2.8$  kJ mol<sup>-1</sup>, an improvement on previous studies and arguably within the limits of simulation error. In contrast to previous work, methylamine was found to be more hydrophilic than ammonia, although this result was probably aided by the previously discussed problems for ammonia.

Much of the observed free energy behavior may be understood in terms of the molecules' dipole moments; these strongly influence the water structure surrounding the solute molecule. Table IV contains the dipole moments for all 22 molecules with all four charge sets. The general trends are as follows. The restraint slightly decreases the dipole moment of 6-31G\* charges by an average of 1.3%. This was expected to have only a small effect on  $\Delta G_{\text{hyd}}$ , but as Table II indicates, the average free energy of hydration became more positive by 1.8 kJ mol<sup>-1</sup>. Switching to the 6-31<sup>+</sup>G\* basis sets increased the dipole moment by an average of 4.0% for EPD charges and by a similar value for REPD charges. By combining the 6-31<sup>+</sup>G\* basis set with the restraint, these two effects cancel to some extent, giving slightly more polarized molecules with OPLS-like charges that reproduce experimental free energies of hydration well.

**TABLE IV.**  
**Dipole Moments (D) for All 22 Molecules from All Four Charge Sets.**

Molecule	$\mu(\text{EPD} / 6-31\text{G}^*)$	$\mu(\text{REPD} / 6-31\text{G}^*)$	$\mu(\text{EPD} / 6-31^+\text{G}^*)$	$\mu(\text{REPD} / 6-31^+\text{G}^*)$
Methane	0.00	0.00	0.00	0.00
Ethene	0.00	0.00	0.00	0.00
Water	2.25	2.22	2.35	2.30
Methanol	2.15	2.10	2.27	2.20
Methanethiol	2.07	2.06	2.12	2.10
Acetaldehyde	3.05	3.02	3.30	3.26
Acetone	3.17	3.16	3.42	3.41
Acetic acid	1.72	1.71	1.86	1.85
Methyl acetate	1.95	1.97	2.07	2.12
Ammonia	1.96	1.90	1.94	1.84
Methylamine	1.82	1.72	1.86	1.72
Acetamide	4.04	4.00	4.30	4.26
<i>trans</i> -N-Methyl acetamide	4.09	4.09	4.29	4.30
Dimethyl ether	1.65	1.64	1.72	1.72
Dimethyl sulfide	1.99	2.00	2.04	2.04
Chloroethane	2.48	2.47	2.53	2.53
Benzene	0.00	0.00	0.00	0.00
Phenol	1.95	1.85	2.01	1.88
Aniline	1.60	1.57	1.58	1.58
Chlorobenzene	2.24	2.23	2.21	2.17
Formaldehyde	2.68	2.66	2.87	2.87
Formamide	4.08	4.03	4.28	4.22

While the value of the restraint used in the REPD charge parameterization was chosen to optimize charges to their OPLS values, the restraint used may conceivably be optimized to reproduce some experimental quantity such as the free energy of hydration. However, this procedure would be very computationally intensive.

## LR

The energy and SASA data calculated from the Monte Carlo simulations of each molecule in water required for the LR parameterization are presented in Table V for the EPD/6-31G\* and REPD/6-31<sup>+</sup>G\* charge sets. The FEP results suggest that these charge sets are the most useful. The statistical errors on  $\langle U_{\text{vdW}} \rangle$  and  $\langle U_{\text{elec}} \rangle$  are standard errors calculated over batch averages of 0.5 M configurations and vary from zero to approximately 2 and 6 kJ mol<sup>-1</sup>, respectively. Comparing the energy data obtained for each molecule, there is no discernable trend between the two charge sets and the SASAs are almost identical.

Table VI contains the results of fitting these energies and SASAs to either experimental or FEP free energies of hydration using eq. (2). The LR

parameters  $\alpha$ ,  $\beta$ , and  $\gamma$  are presented. Also given are the gradients and correlation coefficients of the predicted free energies of hydration versus the free energy data used in the fit. Finally, the average unsigned errors and average cross-validation (CV) errors are listed. The CV error was calculated by dividing each set of molecules into four groups at random, fitting to three of the groups, and applying the resulting LR parameters to the fourth to give an average error. Each of the four groups was examined in turn and the complete procedure repeated 100 times.

The values of LR parameters obtained from the fitting procedure are consistent with those reported elsewhere for free energies of hydration.<sup>7,8,24</sup> In particular, the  $\beta$  values were found to lie in the vicinity of 0.5, but there was more variation for  $\alpha$  and  $\gamma$ . Although the utility of the LR method involves fitting to experimental free energies of hydration, in assessing the performance of the LR method itself, the results of fitting to FEP values should be examined. Because FEP is formally an exact method, the ability of LR to reproduce FEP results is a critical test of this free energy methodology. From Table VI it is apparent that all the LR free energies agree with their equivalent FEP val-

**TABLE V.**  
**van der Waals, Electrostatic Solute – Solvent Energies (kJ mol<sup>-1</sup>), and Solvent Accessible**  
**Surface Areas (Å<sup>2</sup>) for EPD/6–31G\* and REPD/6–31<sup>+</sup>G\* Molecules.**

Molecule	EPD/6–31G*			REPD/6–31 <sup>+</sup> G*		
	$\langle U_{vdW} \rangle$	$\langle U_{elec} \rangle$	SASA	$\langle U_{vdW} \rangle$	$\langle U_{elec} \rangle$	SASA
Methane	–14.32	–0.22	143.4	–13.49	–0.05	143.6
Ethene	–17.77	–9.94	170.3	–16.20	–12.90	170.4
Water	10.60	–85.12	113.6	11.37	–93.24	113.5
Methanol	–7.01	–67.87	160.0	–6.10	–69.78	160.0
Methanethiol	–16.55	–30.55	182.1	–17.58	–28.29	182.0
Acetaldehyde	–19.34	–50.50	188.5	–17.21	–59.29	188.6
Acetone	–26.63	–59.53	219.0	–24.06	–69.43	219.3
Acetic acid	–14.65	–96.03	197.5	–16.07	–87.66	197.4
Methyl acetate	–30.55	–60.54	234.3	–30.64	–62.37	243.3
Ammonia	2.38	–58.28	132.1	1.42	–55.03	132.1
Methylamine	–5.82	–59.33	171.5	–5.26	–61.81	172.0
Acetamide	–15.26	–111.23	204.2	–18.42	–101.13	204.4
<i>trans</i> -N-Methyl acetamide	–29.32	–107.35	239.7	–32.26	–95.19	239.9
Dimethyl ether	–23.14	–33.99	196.5	–20.72	–35.87	196.5
Dimethyl sulfide	–30.22	–16.54	213.8	–30.53	–18.82	214.0
Chloroethane	–28.21	–17.17	177.7	–28.94	–17.84	177.6
Benzene	–38.71	–27.87	244.5	–38.66	–41.41	244.2
Phenol	–36.70	–66.47	255.9	–32.65	–72.72	256.4
Aniline	–34.75	–74.33	265.4	–38.43	–60.96	265.1
Chlorobenzene	–48.56	–17.71	242.7	–49.74	–17.88	242.8
Formaldehyde	–12.88	–40.11	152.7	–12.15	–45.91	152.8
Formamide	–7.95	–111.06	170.5	–10.34	–97.44	170.7

ues to within approximately 2 kJ mol<sup>-1</sup>. REPD/6–31<sup>+</sup>G\* and EPD/6–31G\* are in closest agreement with mean unsigned errors of 1.8 and 1.9 kJ mol<sup>-1</sup>, respectively. However, one would expect the LR parameters obtained for each charge set to be very similar. That this is not the case, particularly for  $\alpha$ , is a cause for concern.

The ability of LR to reproduce experimental free energies of hydration examines the reliability not only of the free energy methodology, but also of the force field. In this regard the EPD/6–31G\* charge set is the most reliable with a mean unsigned error of 2.2 kJ mol<sup>-1</sup>, followed by both REPD charge sets with mean unsigned errors of

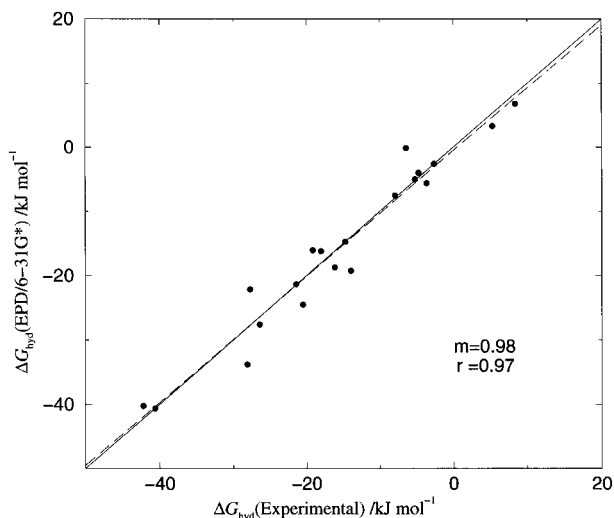
**TABLE VI.**  
**Linear Response Parameters ( $\alpha$ ,  $\beta$ , and  $\gamma$ ), Slopes ( $m$ ), Correlation Coefficients ( $r$ ), Average Errors, and CV Errors for Each Charge Set Fitted Using Eq. (2) to Experiment and FEP.**

$\Delta G$ Source	Charge Set	$\alpha$	$\beta$	$\gamma$	$m$	$r$	Ave. Error	CV Error
Experiment	EPD/6–31G*	0.303	0.467	0.078	0.98	0.97	2.2	2.9
	REPD/6–31G*	0.382	0.506	0.092	0.89	0.94	3.0	3.7
	EPD/6–31 <sup>+</sup> G*	0.366	0.444	0.086	0.95	0.92	3.6	4.7
	REPD/6–31 <sup>+</sup> G*	0.370	0.502	0.093	0.94	0.96	3.1	4.2
	All molecules	0.350	0.471	0.085	0.93	0.94	3.2	3.4
Simulation	EPD/6–31G*	0.678	0.525	0.137	0.97	0.98	1.9	2.2
	REPD/6–31G*	0.582	0.491	0.120	0.90	0.97	2.5	3.4
	EPD/6–31 <sup>+</sup> G*	0.506	0.519	0.115	0.95	0.98	2.3	2.9
	REPD/6–31 <sup>+</sup> G*	0.532	0.551	0.131	1.01	0.98	1.8	2.4
	All molecules	0.594	0.526	0.129	0.96	0.98	2.3	2.4



approximately  $3 \text{ kJ mol}^{-1}$ . These errors are larger than those obtained on fitting to FEP, as would be expected because the reliability of the LR relationship and the force field both affect the fit. It is noteworthy that the LR parameters obtained on fitting to FEP are generally larger than those obtained on fitting to the experiment, particularly for  $\alpha$  and  $\gamma$ . The predicted free energies of hydration for EPD/6-31G\* and REPD/6-31G\* are given in Table VII, together with experimental data. The results are also presented in graphical form in Figure 3 for EPD/6-31G\* charges. The errors given in the last two columns of the table correspond to the differences in free energies of hydration between the LR and the experiment. The free energies of hydration predicted for a number of molecules are particularly poor, supporting the notion that either the force field or the LR methodology is failing in certain cases and that, moreover, these failures may detrimentally affect the overall LR fit.

The mean unsigned errors for the LR method fitted to the experiment are smaller than the equivalent errors for the FEP calculations reported in



**FIGURE 3.** LR EPD / 6-31G\* free energies of hydration fitted using eq. (2) versus the experiment for the molecules listed in Table VII. (---) The line of best fit and the (—) unit slope.

**TABLE VII.**  
Predicted LR Free Energies of Hydration ( $\text{kJ mol}^{-1}$ ) Fitted to Experiment Using Eq. (2)  
for EPD / 6-31G\* and REPD / 6-31G\* Charge Sets.

Molecule	Experiment	$\Delta G_{\text{hyd}}$		Error	
		EPD / 6-31G*	REPD / 6-31G*	EPD / 6-31G*	REPD / 6-31G*
Methane	8.4	6.8	8.4	1.6	0.0
Ethene	5.3	3.3	3.5	2.0	1.8
Water	-26.4	-27.6	-32.0	1.2	5.6
Methanol	-21.4	-21.3	-22.3	-0.1	0.9
Methanethiol	-5.2	-5.0	-3.7	-0.2	-1.5
Acetaldehyde	-14.7	-14.7	-18.5	0.0	3.8
Acetone	-16.1	-18.7	-23.2	2.6	7.1
Acetic acid	-28.1	-33.8	-31.5	5.7	3.4
Methyl acetate	-13.9	-19.2	-19.9	5.3	6.0
Ammonia	-18.0	-16.1	-14.8	-1.9	-3.2
Methylamine	-19.1	-16.0	-16.9	-3.1	-2.2
Acetamide	-40.6	-40.6	-38.5	0.0	-2.1
<i>trans</i> -N-Methyl acetamide	-42.2	-40.2	-37.3	-2.0	-4.9
Dimethyl ether	-7.9	-7.5	-7.3	-0.4	-0.6
Dimethyl sulfide	-6.4	-0.1	-0.7	-6.3	-5.7
Chloroethane	-2.6	-2.6	-3.1	0.0	0.5
Benzene	-3.6	-5.6	-12.3	2.0	8.7
Phenol	-27.7	-22.1	-24.6	-5.6	-3.1
Aniline	-20.5	-24.5	-20.0	4.0	-0.5
Chlorobenzene	-4.7	-4.0	-4.7	-0.7	0.0
Formaldehyde	—	-10.7	-13.3	—	—
Formamide	—	-40.9	-36.8	—	—

Table I with the exception of the REPD/6-31<sup>+</sup>G\* results for which FEP is still better. This suggests that the LR parameterization is able to reduce the errors in predicted free energies arising from inadequacies in the force field.

The CV errors presented in Table VI give an indication of the sensitivity of the LR parameters to the molecules used in the fit. This sensitivity is revealed by the degree to which the CV error is worse than the average unsigned error. For the individual charge sets the CV errors given in Table VI are larger than the average error by between 0.3 and 1.1 kJ mol<sup>-1</sup>. These CV errors suggest that more molecules should be included in the fitting procedure, especially when fitting to an experiment. Consequently, the parameterizations were repeated using all the molecules from every charge set. In this way, the influence of a few poorly reproduced molecules would be reduced and indeed the resulting CV error was now only marginally worse than the average. The LR parameters obtained by fitting simultaneously to all charge sets lie in the range spanned by each individual charge set.

As already indicated, the values of  $\alpha$  obtained from the fitting procedure vary significantly between charge sets and this is particularly true when fitting to FEP free energies of hydration. This result is unexpected and raises the possibility that eq. (2) may in fact be overfitting to the data. In this regard, the strong correlation between  $\alpha$  and  $\gamma$  observed here (correlation coefficient of 0.96) and noted elsewhere<sup>7</sup> is of particular concern.

To gauge the importance of the three molecular quantities ( $\langle U_{vdW} \rangle$ ,  $\langle U_{elec} \rangle$ , and SASA) used in eq. (2) a procedure involving the randomization of each of the molecular quantities was employed. In this method the values of a given quantity are reassigned at random to each molecule while the remaining two terms are unaltered. A fit is then performed using eq. (2) and the average error and correlation coefficient calculated. This procedure was repeated 100 times and the overall average error and correlation coefficient obtained. If the calculated free energies of hydration are strongly dependent on one of these quantities, then the fit would be expected to become extremely poor. On the other hand, if the fit is largely unaffected, it would suggest that use of that particular quantity in the fitting equation is unjustified. The results for fitting EPD/6-31G\* and REPD/6-31<sup>+</sup>G\* energy data with FEP free energies of hydration using eq. (2) are presented in Table VIII. It is evident that when  $\langle U_{elec} \rangle$  is randomized, the average error in-

**TABLE VIII.**  
Average Errors and Correlation Coefficients Obtained when Fitting to FEP Using Eq. (2) with Randomized  $\langle U_{elec} \rangle$ ,  $\langle U_{vdW} \rangle$ , and SASA.

Quantity	EPD/6-31G*		REPD/6-31 <sup>+</sup> G*	
	Ave. Error	<i>r</i>	Ave. Error	<i>r</i>
$\langle U_{vdW} \rangle$	3.6	0.94	3.5	0.94
$\langle U_{elec} \rangle$	9.6	0.62	8.9	0.58
SASA	2.8	0.97	3.0	0.96

creases dramatically and the correlation of predicted free energy versus FEP is severely degraded. However, when  $\langle U_{vdW} \rangle$  and SASA are randomized, the average error and correlation coefficient increase much less significantly. These results suggest that although the energy terms and SASA used in eq. (2) have some systematic influence on the free energies, the contribution to the total free energy from  $\langle U_{vdW} \rangle$  and SASA may not be properly resolved. This would be particularly true when there is more noise in the fit as when, for example, a smaller number of molecules or shorter simulations are used. Nevertheless, the electrostatic term appears to be always the most significant by far.

A range of other LR functions were examined for fitting EPD/6-31G\* energy data to FEP free energies of hydration. A similar analysis was performed by McDonald et al.<sup>8</sup> The results given in Table IX indicate that reducing the number of parameters in the LR relationship does indeed lower the predictive ability of the function, as would be expected. However, a number of these relationships perform almost as well as eq. (2) but with either fewer parameters, energy terms, or, indeed, both. Two equations of particular interest are the following:

$$\Delta G = \alpha \langle U_{vdW} \rangle + \beta \langle U_{elec} \rangle + \gamma \tag{3}$$

and

$$\Delta G = \alpha + \beta \langle U_{elec} \rangle. \tag{4}$$

The SASA term was originally included to allow the possibility of positive free energies of hydration.<sup>7</sup> Equations (3) and (4) also allow for this possibility by virtue of  $\gamma$  and  $\alpha$  being positive in each case. In eq. (3) the SASA term has been replaced by a single parameter,  $\gamma$ , and the resulting fit is only 0.2 kJ mol<sup>-1</sup> worse than that observed for eq. (2). Given that the statistical errors on  $\langle U_{vdW} \rangle$  and  $\langle U_{elec} \rangle$  (data not shown) are gener-

**TABLE IX.**  
**Linear Response Parameters, Average Errors, and CV Errors Fitting to FEP Using Each Function**  
**for EPD/6–31G\* Charge Set.**

Function	$\alpha$	$\beta$	$\gamma$	Error	CV Error
$\alpha\langle U_{\text{vdW}} \rangle + \beta\langle U_{\text{elec}} \rangle + \gamma(\text{SASA})$	0.678	0.525	0.137	2.2	2.9
$\alpha + \beta\langle U_{\text{elec}} \rangle + \gamma(\text{SASA})$	1.471	0.424	0.030	3.7	4.1
$\alpha\langle U_{\text{vdW}} \rangle + \beta\langle U_{\text{elec}} \rangle + \gamma$	0.202	0.448	14.03	2.1	3.1
$\alpha\langle U_{\text{vdW}} \rangle + \beta\langle U_{\text{elec}} \rangle$	−0.042	0.337	—	4.6	5.8
$\beta\langle U_{\text{elec}} \rangle + \gamma(\text{SASA})$	—	0.426	0.039	3.9	4.6
$\alpha + \beta\langle U_{\text{elec}} \rangle$	9.098	0.446	—	2.8	3.1
$\beta\langle U_{\text{elec}} \rangle$	—	0.331	—	4.7	5.3

ally larger than this, the question must be asked as to whether the inclusion of a SASA term is statistically significant. In eq. (4) a two parameter equation is proposed involving only  $\langle U_{\text{elec}} \rangle$ . The increase in error of  $0.9 \text{ kJ mol}^{-1}$  is of the same order of magnitude as the statistical errors in  $\langle U_{\text{elec}} \rangle$ . Finally, fitting eq. (4) to FEP for all four charge sets in turn yields values that range from 9.10 to 10.04  $\text{kJ mol}^{-1}$  for  $\alpha$  and from 0.439 to 0.459 for  $\beta$ . These parameters are much more consistent than those derived from eq. (2) and reported in Table VI. Because fitting to FEP free energies of hydration removes any systematic influence arising from the different nonbonded parameters, one would expect the LR parameters obtained for each charge set to be virtually identical. That this is the case for eq. (4) is gratifying. A physical interpretation of eq. (4) is that the  $\alpha$  term corresponds to some averaged free energy of hydration for a solute with charge parameters of zero. Indeed, the free energies of hydration of methane, ethane, and propane are all approximately  $8 \text{ kJ mol}^{-1}$ ,<sup>25</sup> close to the values of  $\alpha$  obtained on fitting to FEP.

## Conclusion

To determine the reliability of the REPD charge calculation method described in our previous study,<sup>1</sup> the free energies of hydration of 22 simple organic molecules were calculated using FEP methods and LR theory. The results obtained from the FEP calculations must be considered the more reliable for assessing the quality of the electrostatic parameters because the method is theoretically exact. The LR procedure, on the other hand, is parameterized through fitting to either experimental or theoretical free energies of hydration and the quality of the calculated free energies therefore depends not only on the force field parameters,

but also on the choice of LR relationship and fitting procedure. The results of the FEP calculations indicate that REPD/6–31<sup>+</sup>G\* charges are the most reliable for the molecules examined, giving an average unsigned error with respect to the experiment of  $2.9 \text{ kJ mol}^{-1}$ . EPD/6–31G\* charges are the next most reliable with an average error of  $3.5 \text{ kJ mol}^{-1}$ . These calculations therefore support the use of the REPD charge calculation procedure at the 6–31<sup>+</sup>G\* level for condensed phase computer simulations.

Use of the three parameter LR equation recommended by Carlson and Jorgensen<sup>7</sup> gave good agreement with the experiment. The EPD/6–31G\* charge set gave an average absolute error with respect to the experiment of  $2.2 \text{ kJ mol}^{-1}$ , whereas the REPD/6–31<sup>+</sup>G\* charge set gave an average error of  $3.1 \text{ kJ mol}^{-1}$ . The average absolute errors obtained on fitting to the experiment are generally smaller than their FEP equivalents, suggesting that LR is able to reduce the errors in the predicted free energies arising from inadequacies in the force field.

Closer inspection of the LR parameters obtained on fitting to FEP revealed an unexpectedly wide spread in values. Fitting to FEP should eliminate all systematic differences arising from the different parameter sets because the FEP results also include these effects, and thus the LR parameters obtained should be almost identical. Given that a close correlation between  $\alpha$  and  $\gamma$  was observed, randomization of  $\langle U_{\text{vdW}} \rangle$  and SASA had only a small detrimental effect on the quality of the fit, and some simpler LR relationships gave only marginally worse performance, the possibility must be considered that eq. (2) may be overfitting to the data. A detailed statistical analysis of the LR method is clearly necessary, and this work is currently in progress.

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